Remarks

Reconsideration of this Application is respectfully requested.

Upon entry of the foregoing amendment, claims 2-4 and 11-13 are pending in the application, with claims 1-3 being the independent claims. Claims 12 and 13 are sought to be added. Claims 5-7, being drawn to nonelected inventions, were previously cancelled without prejudice to or disclaimer of the subject matter therein. In the Amendment and Reply filed December 22, 2008, claims 8-10 were cancelled without prejudice to or disclaimer of the subject matter therein, and claim 11 was added. These changes are believed to introduce no new matter, and their entry is respectfully requested.

Support for the amendment to claims 2 and 3 appears, for example, at paragraph [0065] in the application as filed. Support for new claim 12 appears, for example, at paragraphs [0017] and [0018] in the application as filed. Support for new claims 13 appears, for example, at paragraphs [0066] and [0067] in the application as filed.

Based on the above amendment in conjunction with the prior Amendment and Reply filed on December 22, 2008, Applicants respectfully request that the Examiner reconsider all outstanding objections and rejections of the Office Action dated September 26, 2008, and that they be withdrawn.

Rejections under 35 U.S.C. § 102

Claims 1 and 8 were rejected under 35 U.S.C. § 102(b) as being anticipated by EP 0 979 868 A2 to Kreader *et al.* ("Kreader"). Claims 1 and 2 were rejected under 35 U.S.C. §102(b) as being anticipated by Physica A (1998) 249(1): 216-225 to Gorelov *et al.* ("Gorelov"). Claims 1 and 8 have been cancelled, rendering their rejection moot. Applicants therefore respectfully request the rejection of these claims be withdrawn.

Claim 2 has been amended to recite "adding a cationic surfactant to a sample that contains a nucleic acid and contains a substance that is to be separated from said nucleic acid, wherein said cationic surfactant is added in an amount sufficient to adjust the electric charge of said substance so that said charge is more positive than before the adjustment." Claim 2 has also been amended to recite "as a result of said electrophoresing in the presence of said cationic surfactant, said substance migrates further in a direction opposite to said nucleic acid than it does without the presence of said cationic surfactant."

Gorelov does not disclosed this feature. The Examiner asserts that Gorelov teaches a cationic surfactant interacts with DNA by displacing positively charged bound counterions (i.e., sodium counterions) (see pages 223-224 of Gorelov). However, Applicants respectfully draw Examiner's attention to Gorelov's statement that:

> The binding of the surfactant causes a sharp decrease in the electrophoretic mobility in the cooperative part of binding with continuing decrease at saturation.

Decreasing the mobility of the DNA is not the effect of the invention. Applicants respectfully draw Examiner's attention to Applicants' Figure 1 - in which it is shown that the surfactant does not attach to the DNA in Applicants' procedure.

Moreover, Applicants note that Gorelov had to specifically degrade his DNA prior to the addition of the surfactant so that he could obtain fragments of a sufficiently small size so that they did not precipitate from solution in the presence of the surfactant.

In Applicants' method, it is not required that the DNA be degraded before electrophoresis. Also, it is not required that the surfactant bind to the DNA itself. In fact, neither of these requirements of Gorelov is desired (see Gorelov page 217, last two sentences before section 2; also see page 218, starting with line 1, wherein Gorelov teaches how he sonicated his DNA in order to obtain short fragments).

Additionally, Gorelov's electrophoresis sample contains only DNA, HEPES buffer, EDTA and surfactant (Gorelov p. 219, section 2.4). Gorelov does not disclose a sample that also contains a substance that is to be purified from the DNA.

Accordingly, Gorelov does not anticipate the claimed invention. Applicants therefore respectfully request this rejection of claim 2 be withdrawn.

Rejections under 35 U.S.C. § 103

Gorelov in view of Irie

Claim 3 was rejected under 35 U.S.C. § 103(a) as being unpatentable over the Gorelov publication in view of U.S. Patent No. 6,387,235 to Irie *et al.* ("Irie").

Claim 3 has been amended to recite "adding a cationic surfactant to a sample that contains a nucleic acid and contains a substance that is to be separated from said nucleic acid, wherein said cationic surfactant is added in an amount sufficient to adjust the electric charge of said substance so that said charge is more positive than before the adjustment." Claim 3 has also been amended to recite "electrophoresing said sample to concentrate and purify the nucleic acid, wherein, as a result of said electrophoresing in the presence of said cationic and nonionic surfactants, said substance migrates further in a direction opposite to said nucleic acid than it does without the presence of said cationic and nonionic surfactants."

As noted above, Gorelov does not disclose a sample that also contains a substance that is to be purified from the DNA. Accordingly, Gorelov does not disclose concentrating and purifying a nucleic acid using electrophoresis wherein a substance other than the nucleic acid migrates further in a direction opposite to said nucleic acid than it would without the presence of said cationic and nonionic surfactants, as provided by claim 3. Moreover, Gorelov notes that the binding of surfactants to DNA is usually followed by phase separation or, in the case of very high molecular weight DNA in very dilute solutions, by chain collapse. Gorelov was forced to break the DNA in his sample into a small size and also - to use very dilute solutions of DNA to avoid this problem.

This is contrary to the result of the electrophoresis in the claimed method in which the DNA is ultimately concentrated, and, degradation into small fragments prior to addition of the surfactant is not desired and is not required. The restrictions on the DNA size that Gorelov was required to use would destroy the advantage of the electrophoretic separation of the invention - which can be used with DNA of any size.

Gorelov required an association between the cationic surfactant and the DNA. However, for the reasons above, the claimed invention does not require such an association. It can be noted that for example, as exemplified, in Figure 1, no surfactant is shown associated with the DNA in the sample. As exemplified in Figure 1, surfactant is only associated with the substance that is to be purified from the nucleic acid by causing it to migrate further in a direction opposite to that of the nucleic acid by changing the charge on that substance. Changing the charge on the DNA by associating the DNA with the cationic surfactant as Gorelov does, would be counteractive to the goal of the invention of enhancing the separation of the substance from the nucleic acid.

Moreover, Gorelov repeatedly states that the electrophoretic mobility of his DNA decreased as a result of binding of the surfactant. A decrease in the mobility of the DNA is also contrary to the effect of the invention as it would counter the movement of the contaminant. The artisan who read Gorelov would be led away from designing a method in which the conditions were such that the DNA was not required to be of a small size, and such DNA remained in solution in electrophoresis in the presence of surfactants, and such surfactants were not required to bind to the DNA itself.

Irie does not disclose or suggest the addition of a cationic surfactant that is present during the electrophoretic separation that causes a non-nucleic acid substance to migrate further in a direction opposite that of the nucleic acid than it would in the absence of said cationic surfactant. Irie is focused on the collection and fractionation of the nucleic acid at the conclusion of that fraction's electrophoresis. In contrast, Applicants' invention is focused on events that happen during the electrophoresis - not on the ultimate collection of nucleic acid. Irie's starting preparation does not contain a non-

nucleic acid substance that Irie desires to separate from the nucleic acid. The nucleic acid in Irie's preparation all migrate in the same direction. Irie is silent on any substance that migrates in a direction opposite the nucleic acid.

As noted by Examiner, at column 9, Irie discloses an embodiment in which Tween 20, a neutral surfactant, is added to the sample buffer. However, in contrast, the claimed invention also recites that a cationic surfactant is present, a feature not used or suggested by Irie as Irie's fractionation of one nucleic acid from another nucleic acid would not benefit from the same.

As shown above, Irie also does not cure the deficiencies of Gorelov. The articulated reasoning that grounded Examiner's conclusions of obviousness can be revisited. *Prima facie* obviousness is not established. Applicants therefore respectfully request this rejection of claim 3 be withdrawn.

Sheldon and Asai

Claims 1-4 were rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. Patent No. 6,129,828 to Sheldon, III *et al.*

("Sheldon") in view of U.S. Patent No. 6,165,758 to Asai ("Asai").

Claim 1 has been cancelled, rendering its rejection moot.

Claim 3 has been amended to recite: "electrophoresing said sample to concentrate and purify the nucleic acid, wherein, as a result of said electrophoresing in the presence of said cationic and nonionic surfactants, said substance migrates further in a direction opposite to said nucleic acid than it does without the presence of said cationic and nonionic surfactants." This features is not disclosed by Sheldon.

In particular, Sheldon teaches using a trap, which assumes the contaminant is moving in the same electrical direction as the nucleic acid. Sheldon does not teach or suggest adjusting the charge of the contaminant so that it electrophoretically migrates in a direction opposite that of the nucleic acid.

Note Sheldon column 6, lines 36-39 in which Sheldon states, "Therefore, the desired substance and the undesired substances of similar charge will move towards the affinity material which is between the first and second chambers" (emphasis added). Sheldon's approach is to capture the desired substance with an affinity capture to pull it away. Sheldon then elutes the desired substance from the affinity material (col. 6, lines 55-58).

However, in contrast, Applicants have found that they can alter the charge on the undesired substance by the addition of cationic surfactant - and now the undesired substance migrates in a direction opposite that of the nucleic acid, and thus separates itself from the nucleic acid further than it would have done in the absence of the cationic surfactant. Applicant's approach to the problem of co-migrating contaminants is completely different from that of Sheldon, and, in fact, could be used with the affinity method of Sheldon if it was desired to do so.

Asai does not cure the deficiencies of Sheldon. As noted by Examiner, Asai uses a cationic surfactant to precipitate a protein contaminant (a deacetylase) from a different protein (CC acylase). However, in the invention, the contaminant is not precipitated.

Additionally, Asai starts with a preparation in which nucleic acid has already been removed - for example see Asai Example 1, column 5, lines 31-34. Therefore, Asai has not addressed, and cannot address the problem of how to separate nucleic acid materials from contaminants that, before the invention, migrate relatively closely together in electrophoretic condition. It is not a solution to Applicants' problem to do what Asai did - that is, to precipitate the undesired substance.

The combination of Skeldon with Asai, at best, might lead the artisan of ordinary skill in the art to attempt to precipitate the contaminant prior to electrophoresis and then to use an affinity capture for the nucleic acid during the electrophoresis. The discussion above has demonstrated that the articulated reasoning that grounded Examiner's conclusions of obviousness can be revisited. The combination of art does not render the

invention *prima facie* obvious. Applicants therefore respectfully request this rejection of claim 3 be withdrawn.

Claim 4 depends from and adds features to independent claim 3; therefore, this claim is patentable for at least the same reasons as described above with respect to claim 3. Applicants therefore respectfully request the rejection of these claims be withdrawn.

Kreader and Helenius

Claims 2-4, 9 and 10 were rejected under 35 U.S.C. § 103(a) as being unpatentable over the Kreader reference in view of Helenius *et al.* (Proceedings of the National Academy of Sciences, USA (1976) 74(2):529-532 ("Helenius").

As provided by independent claims 2 and 3 as amended, a sample that contains a nucleic acid is placed in an electric field for electrophoresis to concentrate and purify the nucleic acid. This is achieved by adding a cationic surfactant (claim 2) to the sample or by adding a cationic surfactant and a nonionic surfactant (claim 3) to the sample in amounts sufficient to adjust the electric charge of a substance in said sample other than said nucleic acid to be more positive than before the adjustment. The adjustment increases the charge difference between the substance and the nucleic acid, and this difference becomes so large that the nucleic acid is efficiently separated from the substance.

In particular, in claim 2, as a result of the electrophoresing in the presence of the cationic surfactant, the substance other than the nucleic acid migrates further in a direction opposite to the nucleic acid than it would without the presence of the cationic surfactant. In claim 3, as a result of the electrophoresing in the presence of the cationic and nonionic surfactants, the substance other than the nucleic acid migrates further in a direction opposite to the nucleic acid than it would without the presence of the cationic and nonionic surfactants. Thus, the substance, which migrates toward a negative electrode, is separated further form, and thus is less of a contaminant, of the electrophoresed nucleic acid, which migrates toward a positive electrode.

With respect to the rejection of independent claims 2 and 3 based on Kreader and Helenius, in the Office Action, the Examiner asserts that Kreader teaches every feature of these claims but for the use of surfactants. The Examiner asserts that Helenius teaches adding surfactants to alter the electrophoretic mobility of proteins, and that it would have been obvious from the teachings of Helenius to add surfactants to the mixture of Kreader to achieve the claimed method of purifying a nucleic acid.

In the Advisory Action mailed on January 13, 2009, the Examiner further asserted that the application of the teachings of Helenius to alter electrophoretic mobility of proteins would not change the principle of operation of the Kreader method, "since using the mixture of surfactants taught by Helenius would serve the same purpose as the low pH condition taught by Kreader, namely alteration of the electrophoretic charge of an impurity in a sample to permit separation of the impurity from nucleic acids."

Applicants respectfully disagree. Kreader and Helenius cannot be combined so as to render the invention obvious such that an artisan of ordinary skill would arrive at the claimed invention. The method of Kreader achieves nucleic acid purification by separating the proteins from the nucleic acids. Kreader specifically discloses the use of low pH conditions for electrophoresis of nucleic acids to achieve this purification, because "**most** nucleic acids and proteins will have opposite charges under acid conditions," (emphasis added) and because "the nucleic acids would be more stable than at neutral pH." *See* paragraphs [0010]-[0012] of Kreader. Note that Kreader cannot separate nucleic acids and proteins that have a similar charge at acidic pH - the discovery of how to separate nucleic acids and contaminants that have a similar charge at any desired pH is the heart of the invention.

Kreader also specifically discloses that the low pH approach "differs from current procedures for preparative electrophoresis of nucleic acids, which are run at a pH where nucleic acids and proteins migrate in the same direction (towards the anode)." *See* paragraphs [0018] of Kreader. Thus, the principle operation of Kreader is not merely

"alteration of the electrophoretic charge of an impurity in a sample to permit separation," as asserted by the Examiner, but is separation at a low pH, *instead of neutral*, in order to achieve *complete* separation and *stabilize* the nucleic acid during the purification process. *See* paragraph [0021] of Kreader. But, even so, Kreader cannot separate nucleic acids from proteins that have a similar charge at an acidic pH. As stated in Kreader paragraph [0031], 5% of the protein in Kreader's electrophoresed sample was found at the anode (i.e., with the nucleic acid), even when electrophoresing at an acidic pH.

Modification of the method of Kreader to use surfactants as taught by Helenius would create *basic* pH conditions, and therefore would change the principle of operation of the Kreader method. Redesigning the method of Kreader such that electrophoresis is not run under acidic conditions would change the basic principle under which the method was Kreader was designed to operate, which is *separation by electrophoresis under acidic conditions*.

Further, based on teachings of Gorelov, the artisan who read Helenius would not be motivated to add a surfactant to the protein/DNA mixture of Kreader to achieve separation of the protein from nucleic acids by migration toward opposite electrodes. Nothing in Helenius suggests the surfactants can be added to a mixture of DNA and proteins to obtain separation. As noted above, Gorelov discloses that the surfactants can bind to DNA, which may be followed by phase separation or chain collapse, and can cause a sharp decrease in the electrophoretic mobility of the DNA. The claimed invention does not require such an association of the DNA with the surfactant, and decreasing the mobility of the DNA would be counteractive to the goal of the invention of enhancing the separation of the substance from the nucleic acid.

For at least the foregoing reasons, Helenius cannot be combined with Kreader to render obvious the claimed invention. The above discussion has shown that the articulated reasoning that grounded Examiner's conclusions of obviousness can be revisited. Applicants therefore respectfully request the rejection of these claims be withdrawn.

Claims 9 and 10 have been cancelled, rendering their rejection moot. Previously presented claim 11 depends from and adds features to independent claim 2; therefore, this claim is patentable for at least the same reasons as described above with respect to claim 2. Claim 4 depends from and adds features to independent claim 3; therefore, this claim is patentable for at least the same reasons as described above with respect to claim 3. Applicants therefore respectfully request the rejection of these claims be withdrawn.

New claims 12 and 13

New claim 12 depends from and adds features to independent claim 2; therefore, this claim is patentable for at least the same reasons as described above with respect to claim 2.

New claim 13 depends from and adds features to independent claim 3; therefore, this claim is patentable for at least the same reasons as described above with respect to claim 3.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

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Date: February 26, 2009

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